and (ii) develop a qualitative picture for the folding landscape. These results are compared and contrasted to the results of a nearly identical RNA construct with a 2 nt substitution mutation associated with the genetic disorder dyskeratosis congentia (DKC). As expected, the WT RNA construct ($\Delta G^{\circ}_{WT} = -4.2 \pm 0.2$ kcal/mol) is substantially more stable than the DKC construct ($\Delta G^{\circ}_{DKC} = 0.26 \pm 0.05$ kcal/mol]. The kinetic origin of this differential stability is the result of a substantially increased folding rate constant (~400 times faster) for the WT and a subtle reduction of the unfolding rate constant (~5 times slower).

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Role of Magnesium Ions and Ligand Stacking in the Adenine Riboswitch Folding

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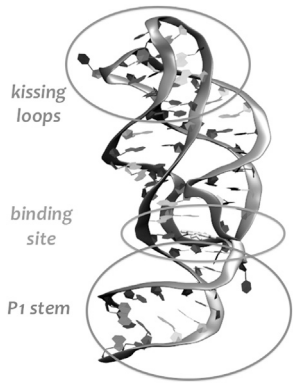
Riboswitches are RNA structured elements that modulate fundamental pathways in bacteria and plants. Depending on the availability of their sensed metabolite they undergo a conformational change that turns on or off gene expression. In the *add* adenine riboswitch from Vibrio Vulnificus there are three crucial spots for the switch between the two conformations: the P1 stem, the kissing-loops and the binding site for adenine. Our work is based on advanced *in silico* techniques, including atomistic molecular simulations, steered molecular dynamics, umbrella sampling, metadynamics and Hamiltonian replica exchange. First we investigated the P1-ligand dependent stabilization quantifying this effect in terms of free energy (Di Palma et al doi:10.1261/rna.040493.113). Then we evaluated the different contributions that Mg^{2+} and adenine give to the formation of the tertiary interactions between the two loops and we analysed the influence of these interactions on the binding site. The obtained data are in quantitative agreement with experiments. The atomistic description of metabolite binding, RNA-Mg^{2+} interaction, and tertiary-contact formation clarifies the details of the ligand-aptamer interactions and of the role of divalent cations in the global aptamer folding.



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Modeling Unpairing Costs for Fast Computation of the Net Binding Free Energy of an Oligo to an mRNA Target

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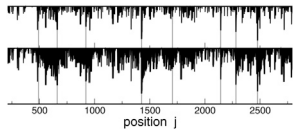
Binding of a small RNA (miRNA, snoRNA, siRNA) often regulates the expression of messenger RNA. The net free energy consists of the contribution of binding, which is well parameterized and fast to compute, offset by the penalty to remove pre-existing mRNA secondary structure, which is $O(N^3)$ to compute with previous methods. Here we introduce a base-composition-dependent model for the mean free energy to open the target which runs in O(N). Implemented in our BINDIGONET algorithm, this model for the net binding free energy of spliceosomal U1-snRNA to real and decoy donor splice sites enhances accuracy.



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Topology of Large RNA Junctions Explored by High-Precision FRET

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Non-protein coding RNAs perform essential functions in living organisms. They commonly exist as dynamic ensembles of conformational states. While many structurally known RNAs are trapped in one or a few conformations by interactions with proteins or tertiary contacts between stems, bulges, or loops the knowledge of equilibrium structures, conformational space, and tertiary conformational changes of large RNAs not being restrained by external or tertiary interactions is still very limited. Helical four-way and three-way junctions (4WJs and 3WJs) are an essential structural motif of the for functional RNA structures. Here we explore the topology of a set of a 4WJ and related 3WJs related to the hairpin ribozyme by measuring more than 250 different FRET-pairs using single-molecule multi-parameter fluorescence detection [1]. Using FRET restrained high-precision structural modeling combined with full atom MD simulations as a hybrid tool [2,3], we resolve the structures of three coexisting conformers of a fully Watson-Crick base paired RNA4WJ. By a suitable choice of the number of bases in the bulges the helices arrangements of the corresponding 3WJs can span a huge conformational space which is necessary for the stem communication in functional RNAs.

[1] Sisamakis, E., et al.; Methods in Enzymology **475,** 455-514 (2010).
[2] Sindbert, S., et al.; J. Am. Chem. Soc. **133**, 2463-2480 (2011).
[3] Kalinin, S. et al. Nat. Methods **9**, 1218-1225 (2012)

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When Freely-Rotating isn't Enough: A Study of Cyanine Dyes on RNA

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Cyanine dyes are commonly attached to the 3' or 5' termini of nucleic acids to study folding and binding using FRET. It has been known for some time now that, to some extent, these dyes stack on the ends of duplex DNA, which complicates the interpretation of FRET. We used molecular dynamics (MD) simulations to study the indocarbocyanine dyes Cy3 and Cy5 attached to the 3' or 5' terminal bases of a 16-base-pair RNA duplex. The resulting trajectories for the inter-dye distance and dye orientation factor (κ^2) are then used to predict FRET for the various RNA constructs. The results show that the average value of FRET depends on both the terminal base and the linker position. In particular, 3' attached dyes typically explore a wide region of configuration space, and the relative orientation factor, κ^2, has a distribution that approaches that of free-rotators. This is in contrast to 5' attached dyes, which spend a significant fraction of their time in one or more configurations that are effectively stacked on the ends of the RNA duplex. However, even for the relatively free 3' attached dyes, the correlation time of κ^2 is still too long to justify the use of a free-rotation approximation.

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Solvent Kinetic Isotope Effects on 2'-Hydroxyl Acylation of RNA

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RNA can fold into complex tertiary structures, much like proteins, and exhibits significant conformational dynamics that are a central facet of its functions. Two limiting types of RNA motions occur: equilibrium fluctuations and induced conformational changes. Equilibrium fluctuations are spontaneous transitions between conformers along the RNA free energy landscape and conformational transitions occur when the free energy landscape is perturbed to generate new minima and change the energy barriers between minima. Surprisingly, little experimental data are available for equilibrium fluctuations for even simple RNA, and even less is known about the driving forces for conformational transitions in large RNAs. In part this is due to limiting technologies. One technology, SHAPE, has overcome many of the limitations for studying large RNAs. SHAPE, for selective 2′-hydroxyl acylation analyzed by primer extension, has revolutionized RNA secondary structure prediction and provides a transformative experimental approach to investigate nucleotide specific equilibrium fluctuations and conformations. SHAPE reactivity is governed primarily by nucleotide flexibility, which in turn is governed by secondary and tertiary structure constraints. In practice, SHAPE provides nucleotide level resolution of RNA structure and dynamics in RNA of any size. We hypothesize that the solvent isotope effect on SHAPE chemistry will allow insights into the roles of hydrogen bonding and solvation on RNA structure and dynamics. Here, we describe the initial experiments and theoretic basis for interpreting this solvent isotope effect.

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Conformational Entropy of the RNA Phosphate-Sugar Backbone

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While major contributors to the free energy of RNA tertiary structures such as base pairing, base stacking and charge and counterion interactions have been studied extensively, little is known about the intrinsic free energy of the backbone. To assess the magnitude of the entropic strains along the